Human Whole Genome DNA Microarray

User Guide

Eurogentec

Notice to the User

It is important that users read the entire manual before commencing work.

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User Guide and Technical Support

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To reach technical support by telephone, call
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Feedback

We welcome your feedback regarding our products and this manual. Please contact us at:
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All comments are welcome.

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Getting Started

Please read the introductory information below to help familiarize yourself with Human Microarray before you begin using it.

Product Contents

- Human Microarray
- Human Microarray Hybridization buffer and tube

  NOTE: Each tube contains buffers sufficient for 5 to 10 microarray hybridization procedures
- Spotted Region Guide
- Human Microarray Product User Guide
- Product Support CD, which contains the following:
  - ⇒ Sample Images folder
  - ⇒ Human Microarray .gal file
  - ⇒ Gene list and probe sequences for Human Microarray
- List of Human Microarray Control Probes
- Microarray layout of Human Microarray
- Electronic version of the Human Microarray Product User Guide

Other Necessary Apparatus and Reagents (Not Supplied)

Apparatus

- Water bath/heating block
- Powder-free gloves
- Clean, blunt forceps
- Micropipettors
- Sterilized and nuclease-free pipet tips
- Sterilized and nuclease-free microcentrifuge tubes
- Microcentrifuge
- Vortex mixer
- Clips and forceps
- Hybridization oven
- Hybridization accessories: chamber cover slides, etc.
- Rectangular slide staining dish and slide rack for washing microarrays
- PCR (polymerase chain reaction) machine
- Microarray scanner for standard 1" x 3" format (see Table 7 on page 21 under "Human Microarray Scanner Specifications" for a list of compatible scanners)
- Hybridization systems (optional)
- Automated hybridization station (optional)

Reagents

- De-ionized nuclease-free water
- Cyanine 3- or 5-labeled amplified aRNA sample, or Cyanine 3- or 5-labeled cDNA sample
- Wash solutions (four types, all necessary):
  - 2X SSPE, 0.1% SDS solution
  - 2X SSPE
  - 0.1X SSPE, 0.1% SDS solution
  - 0.1X SSPE

Reagents (Continued)

- BSA (bovine serum albumin or albumin bovine serum)

NOTE: BSA must be molecular tested.

- Pre-hybridization Buffer:
  - 5X SSPE, 0.1% SDS solution
  - 1% BSA
- Deionized formamide
- Alternatives to Salmon Sperm DNA Blocking Mixtures:
  - Ambion® sheared Salmon Sperm DNA (10 μg/μl), or
  - Invitrogen™ Cot-1 DNA® (2.5 10 μg/μl), or
  - Invitrogen™ Poly-A (2.5 10 μg/μl)

Important Notes on Microarray Handling and Storage

Storage Conditions

- Store Human Microarray product at 4°C.
- Store Human Microarray Hybridization Buffer Tube at room temperature.

NOTE: If the product is received with an open bag, please contact Eurogentec Customer Service for an immediate replacement.

Handling Microarrays

Please read this section carefully and follow the instructions!

- Polynucleotide probes are printed on the side of the slide with the barcode.
- To avoid irreparable damage of the printing area, **do not touch** the surface with bare hands, or with any other objects.

Open arrays should be used within a week.

Product Description and Overview

Human Microarray is made of sense-strand polynucleotide probes spotted onto a proprietary chemical layer coated on top of a 1” x 3” (25 mm x 75 mm) standard-format microarray glass slide. Updated information of human genome content from public domains is used to design greater than 30,000 highly sensitive 60-mer probes for monitoring the expression level of corresponding human protein-coding genes.

Each probe is spotted onto the array in a highly consistent manner using a proprietary, non-contact spotting technology adapted for microarray manufacturing.

Human Microarray Genome Content

Each microarray contains 32,050 oligonucleotides: *30,968 human genome probes*, and *1082 experimental control probes*.

Each oligonucleotide probe is designed to hybridize to a specific target gene described in the current public domain contents, such as UniGene, Cancer Genome Anatomy Project (CGAP), BioCarta, Kyoto Encyclopedia of Genes and Genomes (KEGG), and validated by the Human Genome Sequencing Project (HGSP).

Table 1, below, provides an example of the contents of a human genome that can be studied using the Human Microarray.

Table 1: Human Genome Content	
Probe Type	**Number of Probes**
UniGene and RefSeq based	**30,968 (total)***
UniGene build #175 based and/or RefSeq based with Entrez Gene ID including: **CGAP (Cancer Genome Anatomy Project)** **BioCarts and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways**	**28,703**
UniGene build #163 based with Gene ID and experimentally selected	**2265**

* Human Microarray is guaranteed to print 95% or more of the total probe content.

NOTE: Detailed gene lists, gene annotations, and probe sequences can be found on the Product Support CD that accompanied this product, or on our Web site at:

www.eurogentec.com

Human Microarray Control Features

There are 1,082 control probes built into the Human Microarray that monitor the sample quality and hybridization process. These control probes provide valuable information to ensure experiments are done correctly resulting in higher quality results for analysis.

These control probes are explained in detail in the Step 7 Check Control Probe Data section.

NOTE: Additionally, detailed control probe information can be found on the Product Support CD that accompanied this product, or on our Web site at:

www.eurogentec.com

Using Human Microarray

This section provides you with detailed information about how to perform the steps necessary to complete the hybridization process to study gene expressions using the Human Microarray.

What You Are Going To Do

Follow these detailed steps *exactly* to achieve the best experimentation results.

- **Step 1:** Prepare the RNA Sample
- **Step 2:** Label the Target
- **Step 3:** Pre-Hybridize the Microarray
- **Step 4:** Perform the Hybridization Protocol
- **Step 5:** Wash the Hybridized Microarray
- **Step 6:** Scan and Extract Gene Expression Results
- **Step 7:** Check Control Probe Data

PROCESS COMPLETE!

Step 1: Prepare the RNA Sample

High-quality, intact RNA is essential for all gene expression microarray experiments.

There are many different RNA isolation protocols and commercially-available RNA isolation kits. You should choose a solution that meets your specific needs. Stratagene, Ambion, Invitrogen and other reagent companies offer many different RNA isolation products. For more information, you can visit each company's Web site.

Once the RNA samples are isolated, you must confirm the quantity and quality of the samples. Similarly, many different protocols are available and you should choose a solution that is suitable for your needs.

For faster and more automated RNA analysis, you may want to consider the "No Cuvettes" Spectrophotometer from NanoDrop®, or the 2100 Bioanalyzer from Agilent Technologies. For more information, visit each company's Web site.

Step 2: Label the Target

For best results, it is recommended that you use one of the commercially available labeling kits that has been tested for use with the Human Microarray—please refer to Tables 2 and 3 in the section titled, Step 4B:Prepare Hybridization.

General Guidelines for Target Labeling

There are many commercially-available labeling kits for microarray analysis. Select a labeling kit or labeling method that is most suitable for your specific needs. If you use a labeling kit that is not listed in Tables 2 nor 3 in the Step 4B: Prepare Hybridization section, it is recommended that you validate the method to test and determine its compatibility with the Human Microarray.

You may want to confirm the quality of the labeled target with the "No Cuvettes" Spectrophotometer from NanoDrop®.

RNA Sample Amounts

Generally, the amount needed of quality RNA is 10-20 μg for each labeling reaction.

If you have an *ample* supply of RNA samples, you have the *choice* of using a protocol that either amplifies or does not amplify the RNA sample.

If you have a *limited* amount of RNA samples, it is recommended that you use a protocol that includes a linear amplification of the RNA samples.

Step 3: Pre-Hybridize the Microarray

General Instructions

Human Microrray requires a pre-hybridization step prior to hybridization of the labeled target. The pre-hybridization step reduces background signals and increases the performance of the microarray. Complete the pre-hybridization step by carefully following the instructions below.

1) Pre-hybridization solution: 5X SSPE, 0.1% SDS, and 1% BSA
2) Warm the pre-hybridization solution to 42°C.
3) Carefully and slowly, fully submerge the Human Microarray in an abundant amount of pre-hybridization solution for two hours at 42°C.
4) Transfer the slide(s) to room temperature, distilled water for two minutes.
5) Spin dry the slide(s) for two minutes. It is recommended that you use the slides in the hybridization protocol within one hour of completing the pre-hybridization process.

Instructions Using the Round Cap Tube (Included)

Follow the steps below to complete the pre-hybridization process using the 25 ml Round Cap Tube that holds the Human Microarray.

NOTE: While the round cap tube is included with the Human Microarray product, the pre-hybridization *solution* itself is not.

1) Pre-warm 25 ml of pre-hybridization solution 5X SSPE, 0.1% SDS, and 1% BSA to 42°C.
2) Carefully remove the arrays from the round cap tube. Be sure to remember the original location and direction of the arrays in the tube (see Figure 1).

3) Pour 25 ml of the pre-warmed pre-hybridization buffer solution into the same round cap tube.

4) Carefully and slowly insert the slides back into the tube in exactly the same location and position as they were originally.

Try to insert the slides into the correct position the first time. Avoid inserting and removing the slides more than once from the pre-hybridization buffer solution.

5) After the slides have been inserted properly into the pre-hybridization buffer, screw the cap onto the tube so that it is tightly sealed, invert the tube gently two times and place the tube upright on a stable and flat surface in an oven at 42°C for two hours.

NOTE: It is recommended that the tube be placed on a rack or other stable apparatus to prevent it from accidentally tipping over during this process.

6) After two hours, transfer the slides to the distilled water for two minutes.

7) Spin-dry the slides for two minutes. It is recommended that you use the slides in the hybridization protocol within one hour of completing the pre-hybridization process.

Figure 1, below, provides an illustration of Step 3, the pre-hybridization procedure.

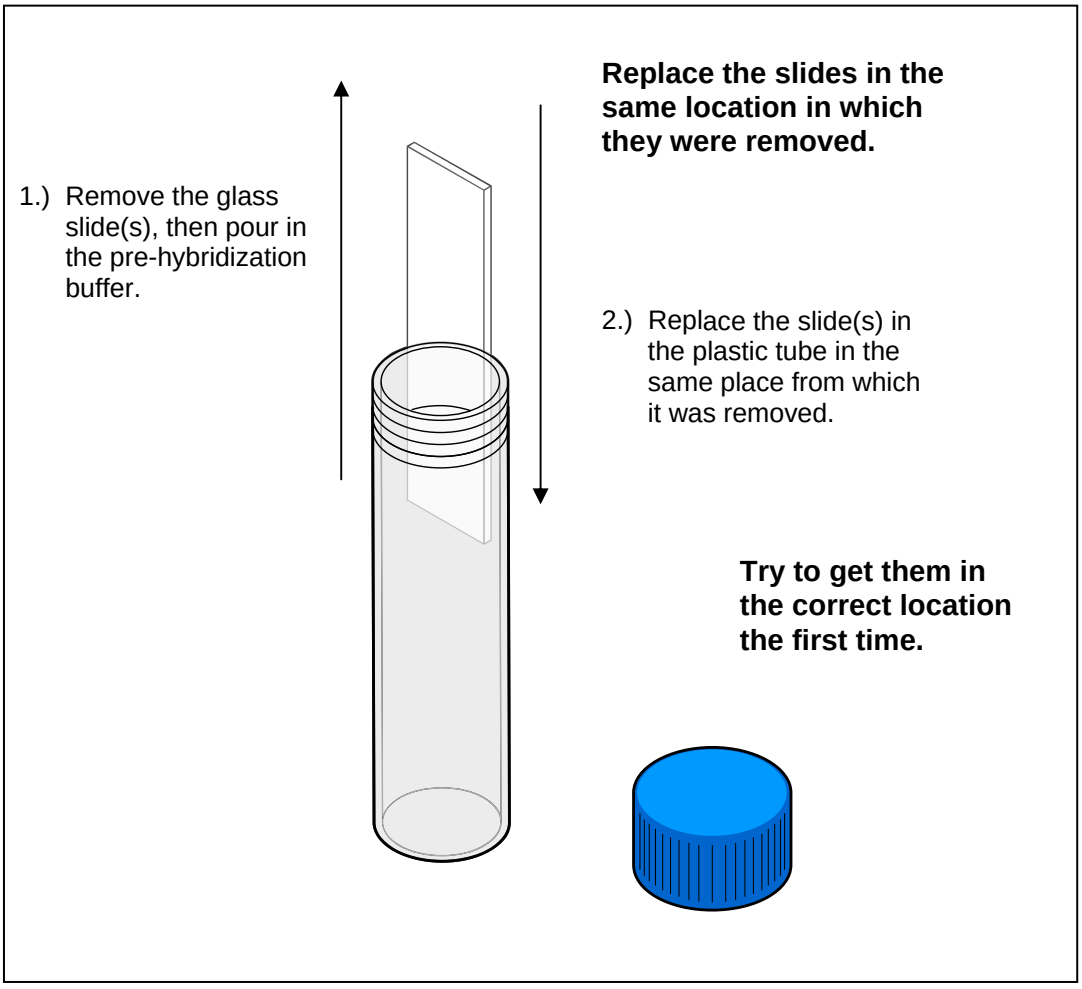
1.) Remove the glass slide(s), then pour in the pre-hybridization buffer.
Replace the slides in the same location in which they were removed.
2.) Replace the slide(s) in the plastic tube in the same place from which it was removed.
Try to get them in the correct location the first time.

Figure 1 Diagram of Round Cap Tube Pre-Hybridization Procedure

Step 4: Complete the Hybridization Protocol

Once you have completed the pre-hybridization step using one of the methods outlined in the Step 3: Pre-Hybridize the Microarray section, you are ready to complete the hybridization protocol.

There are many different hybridization protocols, apparatus, and instruments available that may be compatible for use with the Human Microarray. Detailed instructions for using the glass cover slide method are described below.

For best performance and consistent hybridization results, it is recommended that you use the Human Microarray Hybridization Buffer™, included with this product to complete the hybridization process.

Step 4A:➔Prepare Hybridization Buffer Solution Using the Human Microarray Buffer (Included)

For correct use of this buffer, you must add a specific amount of formamide and labeled target. Please follow the instructions below carefully.

1) Spin down the stock Human Microarray Buffer (~410 µl in each tube).
2) Add 90 µl of deionized formamide.
3) Warm the mixture to 42°C to completely dissolve the solution.
   Yield: 500 µl of 1.5X Hybridization Buffer solution.
4) Aliquot 250 µl of the solution into individual tubes and freeze in darkness at -20°C. Each aliquot is sufficient for five hybridizations using the glass cover slide.

Step 4B:➔Prepare Hybridization

Hybridization Using Labeled Targets from aRNA *or* cDNA Labeling Approaches

Not all RNA labels with the same efficiency for Cy3 and Cy5!

For aRNA Hybridization

Follow the steps below to complete the hybridization process using aRNA.

5) Follow the instructions provided by the reagent supplier. Indirect labeling with NHS ester dye is recommended.

   Table 2, below, contains a list of products that can be used to prepare the labeled aRNA target.

Table 2: aRNA Preparation Products	
Manufacturer	**Product Name and Description**
Ambion®	**Amino Allyl MessageAmp II™ aRNA Kit**
Ambion®	**aRNA Fragmentation Reagent**

2) Mix 2 µg of Cy3-aRNA, or 2 µg of Cy5-aRNA samples with nuclease-free H_20 to yield a final volume of 9 µg.

   NOTE: It is essential to use at least 2 µg of labeled target for each sample.

3) Add 1 µl 10x Fragmentation Buffer, and incubate at 70°C for 15 minutes.

4) Add 1 µl Stop solution, and keep in darkness at -20°C.

For cDNA Hybridization

Follow the steps below to complete the hybridization process using cDNA.

1) Follow the instructions provided by the reagent supplier. Indirect labeling with NHS ester dye is recommended.

   Table 3, below, contains a list of products used to prepare the labeled cDNA target.

Table 3: cDNA Preparation Products

Manufacturer	Product Name and Description
Ambion®	**Amino Allyl MessageAmp II™ cDNA Kit**
Invitrogen™	**Superscript Indirect Labeling System**
Stratagene®	**Fairplay II Microarray Labeling Kit**

2) For cDNA labeling, it is recommended that 15 μg of total RNA be used as starting material.

NOTE: For all cDNA preparation, it is strongly recommended that you re-purify the targets using the Qiagen® Mini-Elute PCR purification column prior to hybridization according to the manufacturer's protocol. Incomplete removal of unincorporated dye from the labeling reaction often contributes to increased background noise. The Qiagen Mini Elute PCR has been found to provide consistently more reliable results.

Step 4C:➔Complete the Hybridization Using the Glass Cover Slide Method

NOTE: If you perform hybridization using methods other than the basic glass cover slide method, it is recommended that you validate the protocol experimentally. For example, the MAUI System from BioMicro Systems, or HS Series of Hybridization Stations from TECAN offer a higher throughput and more automated hybridization methods.

To complete this step, you will need to select a type of glass cover slide. Table 4, below, contains a list of glass cover slides that have been tested and confirmed compatible for use with the Human Microarray Buffer.

Table 4: Compatible Glass Cover Slide Products

Manufacturer	Product Name
BioRad® Laboratories	**SLS 6001 (24x60 mm)**
Erie Scientific Company®	**mSeries LifterSlip™ 25x601-M-5439**

1) Ensure your work and experimentation area, as well as the Human Microarray, are clean before adding the Hybridization Buffer solution to the target array.
2) Thaw and pre-warm the Hybridization Buffer with formamide at 42°C for ten minutes.
3) Prepare the hybridization mix in a 1.5 ml Eppendorf tube according to the Table 5, below.

Table 5: Hybridization Mix Measurements	
For each slide: 55 μl	
Component	***Final Volume***
1.5X Human Microarray Hybridization Buffer	**37 μl**
Sheared Salmon Sperm DNA (10 μg/μl)*	**1 μl**
Target preparation plus nuclease-free ddH_20	**Up to 17 μl**

* Alternatives to Salmon Sperm DNA Blocking Mixtures:
Ambion® sheared Salmon Sperm DNA (10 μg/μl), or
Invitrogen™ Cot-1 DNA® (2.5 10 μg/μl), or
Invitrogen™ Poly-A (2.5 10 μg/μl)

4) Spin down the mixture for five minutes at maximum speed to eliminate to potential debris.
5) Transfer the mixture to a PCR tube.
6) Set a Denature program in the PCR machine as follows:
   95°C Five minutes
   60°C Hold

   Place the hybridization mixture in the PCR machine and run the Denature program. Keep tubes either on the PCR hot plate at 60°C, or on a dry bath at 55°C.
7) Place the "Probe Printed Region Guide" (included) on the table and place the Human Microarray slide, with the printed side up, on top of the Guide, being sure to align it properly with the Guide's markings (see Figure 2).

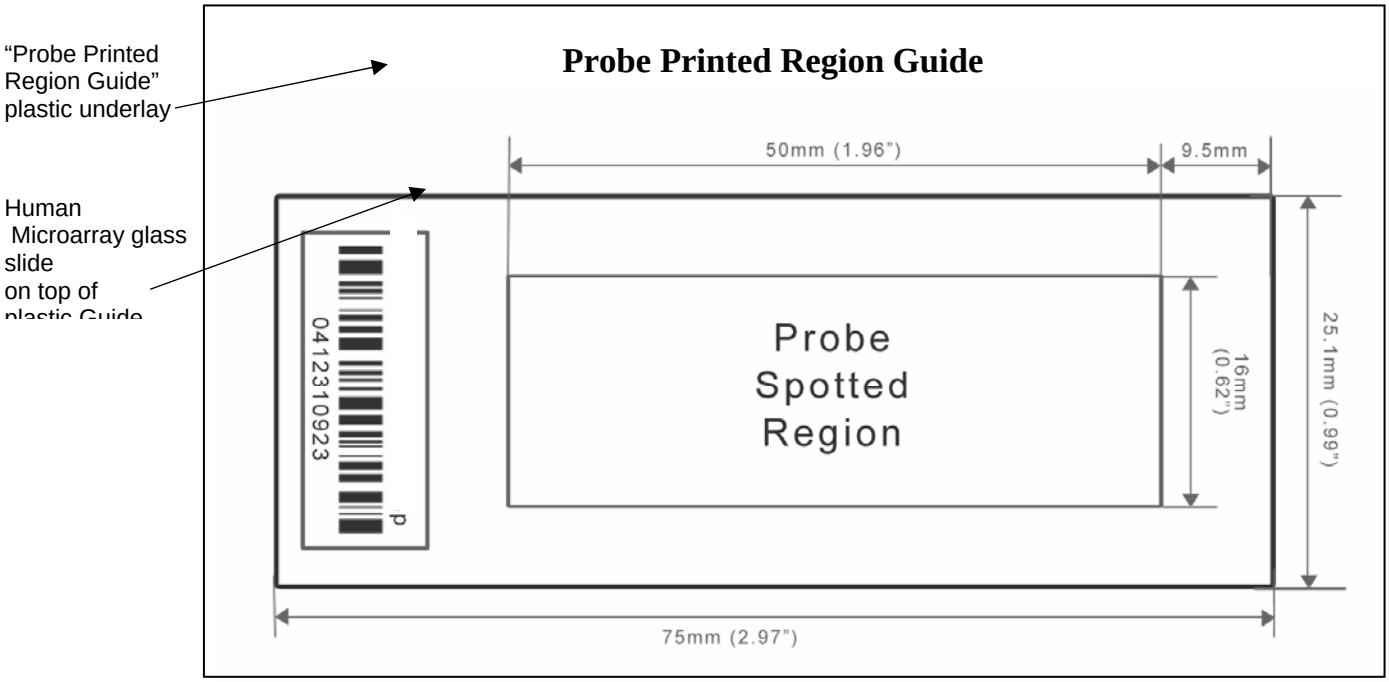

Figure 2: Human Microarray Glass Slide with "Probe Printed Region Guide" Plastic Underlay

8) Pipette the hybridization mixture onto the spotted region of Human Microarray, being sure to avoid creating any bubbles.

9) Carefully place the glass cover slide over the spotted area in an even manner being sure to avoid creating any bubbles.

10) Place the entire labeled target plus the microarray set-up into an closable, *chambered box** that is humidified by 2X SSPE buffer in the 42°C oven for 14 to 16 hours. Ensure that the appropriate humidity level is maintained inside the oven during this 14- to 16-hour period. Figure 3, below, provides an example of this.

Figure 3, below, provides an illustration of Step 4C, completing the hybridization protocol using the glass cover slide method, and specifically, placing the Human Microarray into the chambered box.

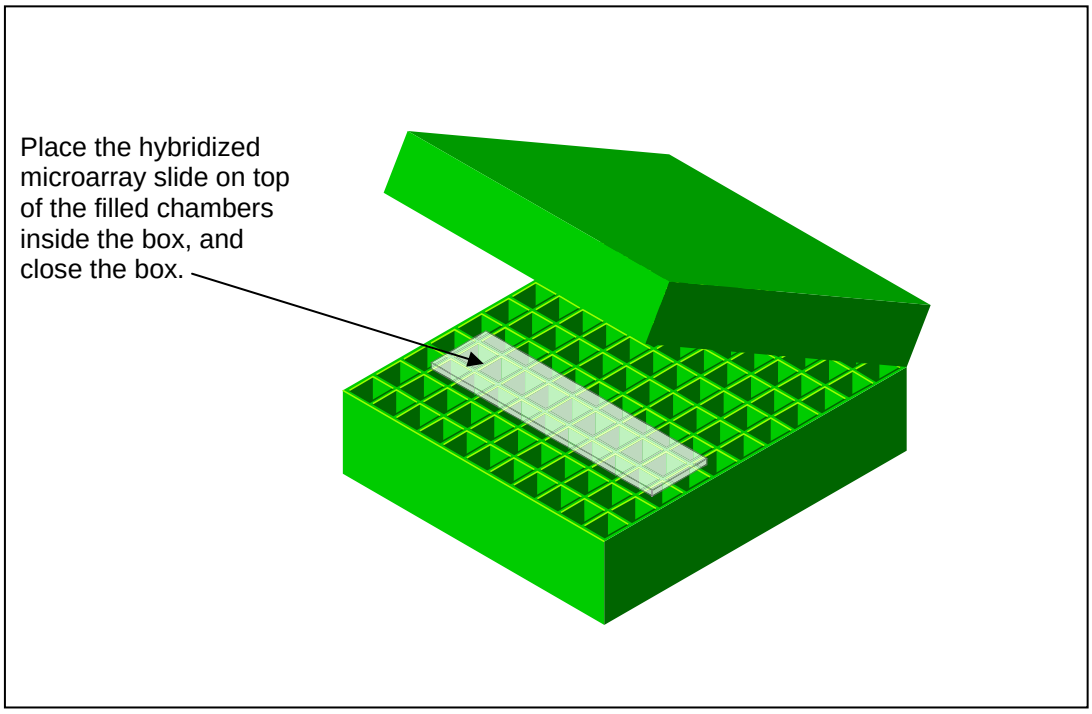

Figure 3: Step 4C → aRNA or cDNA Hybridization—Glass Slide Inside Chamber Box*

*Chambered box—The Hinged 100-Place Storage & Freezer Polypropylene Box from USA Scientific has been used with much success to complete this step. The small (approximately ½ inch x ½ inch) chambers within the box are filled about ¾ full of buffer, then the microarrays are laid on top of the chambers. The box is then closed and placed inside the oven. For information about this product or other USA Scientific products, access their Web site at:

www.usascientific.com

Step 5: Wash the Hybridized Microarray

Washed and dried microarrays should be scanned within a couple of hours.

NOTE: Do not allow the microarray(s) to be exposed to air for a significant amount of time, otherwise, an increased fluorescent background signal could appear.

1) Submerge the entire labeled target and microarray set-up with the cover slide still intact into a large container filled with 42°C 2X SSPE, 0.1% SDS solution.
2) Carefully remove the cover slide from the glass by gently shaking the glass slide so that the cover slide is freed while the slide is submerged.
3) Wash the slide(s) in the "rectangular, slide staining dish and slide rack" with the excess amount of pre-warmed 2X SSPE, 0.1% SDS solution for two minutes at 42°C.
4) One at a time, wash each slide with excess amounts of 0.1X SSPE, 0.1% SDS solution for two minutes at room temperature.

NOTE: When working with multiple microarrays, keep the arrays you are not currently washing in a 2X SSPE solution while they wait for additional processing.

5) Dip each slide into 0.1X SSPE several times at room temperature and rinse using a squeeze bottle.
6) Spin dry with a centrifuge for at least one minute.
7) Keep the microarray dry and in the dark until ready to scan.

Step 6: Scan and Extract Gene Expression Results

There are many scanners available to extract signals from Human Microarray. Data extraction using GenePix™ 4100 from Molecular Devices is described below. For instructions for using other companies' products, refer to information provided by the company.

Table 6, below, lists the setting for using the GenePix 4100.

For a list of scanners that are compatible with the Human Microarray, please refer to Table 6, below.

NOTE: The performance of each scanner may differ. Therefore, to ensure best results, it is recommended that the scanner be adjusted based on standard microarray calibration parameters. Turn on and warm up the scanner for the duration according to manufacture instructions for the scanner.

Use the .gal file and Gene List provided with this product, or refer to our Web site at:

www.eurogentec.com

Table 6: Scanner Settings Using GenePix™ 4100 from Molecular Devices

Wavelength	**635 nm**	**532 nm**
PMT	**680 V**	**640 V**
Minimum diameter (%)	**80**	
Maximum diameter (%)	**180**	
CPI Threshold	**100**	

NOTE: For lower versions of GenePix software, adjust the property parameter to 142.8 µm manually to obtain best results.

Figure 4, below provides a visual example of the Human Microarray glass slide with spotted probe region.

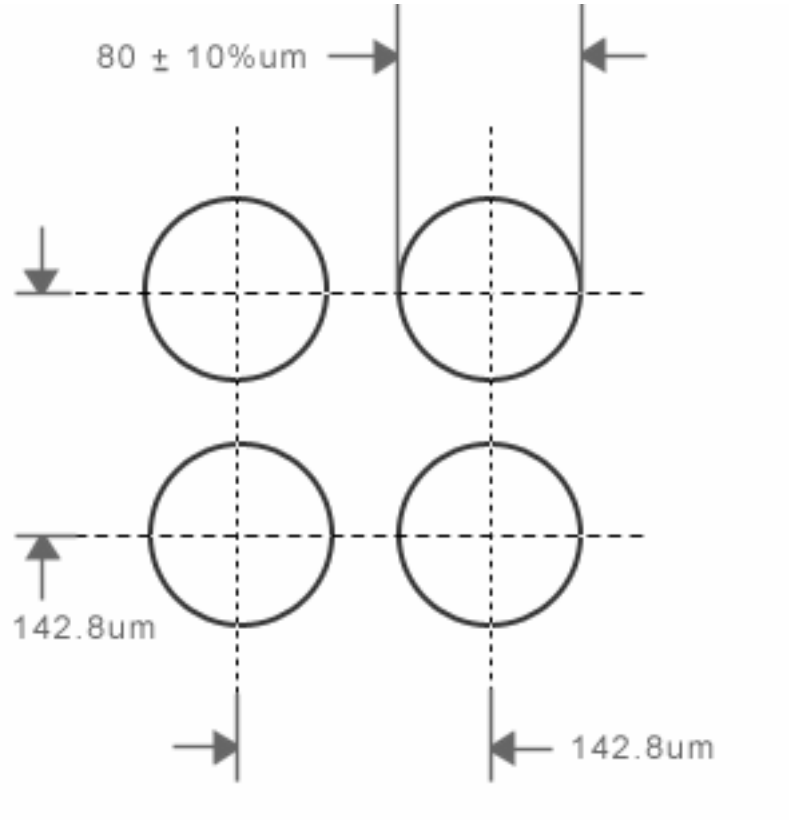

Figure 4: Human Microarray Glass Slide with Spotted Probe Region

Human Microarray Scanner Specifications

Select and use a microarray scanner that meets the specifications below.

Microarray Scanner Specifications

Format capabilities:	**1” x 3” (one inch by three inch) glass slide**
Molecular capabilities:	**Able to accurately detect, activate and read Cy3 and Cy5 fluorescent molecules**

Table 7, below, contains a partial list of microarray scanner products that are compatible for use with the Human Microarray. For more information about the products listed below, refer to each respective company’s Web site.

Table 7: Compatible Microarray Scanners

Manufacturer	Product Name and Description
Molecular Devices	**GenePix® 4000 A/B**
Genomic Solutions,® Inc.	**GeneTAC™ 2000**
Perkin Elmer,® Inc.	**ScanArray™ 5000**
TECAN®	**LS 200/300/400**
Agilent Technology	**DNA Microarray Scanner G2565B**

Step 7: Check Control Probe Data

Human Microarray contains 1,082 built-in control probes for performance monitoring of the hybridization process. They are used to confirm or deny whether the experiment was completed successfully. Details about these probes are explained below.

Additional information about the control probes is included on the Product Support CD, and on our Web site at:

www.eurogentec.com

Human Microarray Control Probes

Orientation Settings and Controls

Orientation Grid Alignment Mark (OGAM)

- Probe ID Number: PH_c_0000089
- Purpose:
  - To define the orientation of the scanned image
- OGAM is a Cy3/Cy5 labeled 60-mer oligonucleotide probe designed from an alien sequence that does not cross-hybridize with human targets. It is located on top of the upper left corner of the print region and serves as an orientation landmark for template registration as different scanners may have different image orientation output formats. Figure 6, below, illustrates this.

Corner Grid Alignment Marks (CGAM)

- Probe ID Number: PH_c_0000072
- Purpose:
  - To define the corners of the scanned image, and to assist with image analysis
- CGAMs are mixtures of Cy3/Cy5 labeled 60-mer oligo designed from an alien sequence that does not cross-hybridize with human targets. CGAMs are located at the four corners of the image and serve as the orientation landmarks for auto or manual grid alignment under Cy3 (532 nm) or Cy5 (635 nm) scanning channel. Figure 5, below, illustrates this.
- Instructions:

  1) Obtain the average and standard deviation of the results in these categories:
     F532 Medium and F532 Mean

Figure 5, below, provides an illustration of the OGAM and CGAM positions.

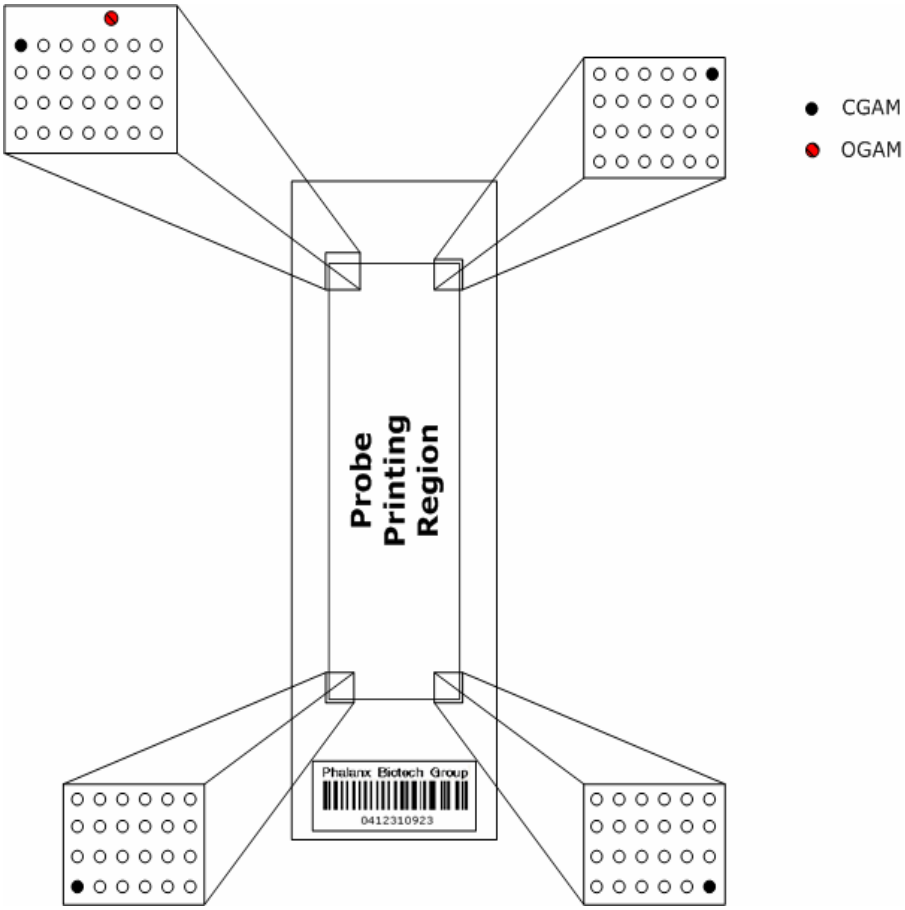

Figure 5: Human Microarray OGAM and CGAM Positions

Grid Alignment Mark (GAM)

- Probe ID Number: PH_c_0000001
- Purpose:
  - To assist in adjusting the scanner settings
  - To monitor the scanner performance across the scanned region
- GAM control probe is a Cy3 labeled 60-mer oligonucleotide probe designed from an alien sequence that does not cross-hybridize with human targets. More than 90 GAM features are evenly distributed across the arrayed area, and these features can be excited by a 532 nm laser without hybridization. GAM probes can serve as orientation or grid-alignment landmarks.
- Instructions:

  1) Convert scanned data into Excel format.
  2) Sort data by column name: ID
  3) Copy ~90 rows of data with Probe ID number: PH_c_0000001
  4) Obtain the medium, average, and standard deviation of the signals in the “F532 Medium” and “F532 Mean” columns.

     Expected Outcome:
     When the suggested scanner settings that are listed in Step 6 are used, saturated or near-saturated signal should be observed for all 90 plus GAM probes.

Figure 6, below, provides and example of scanned Grid Alignment Mark data for the Human Microarray.

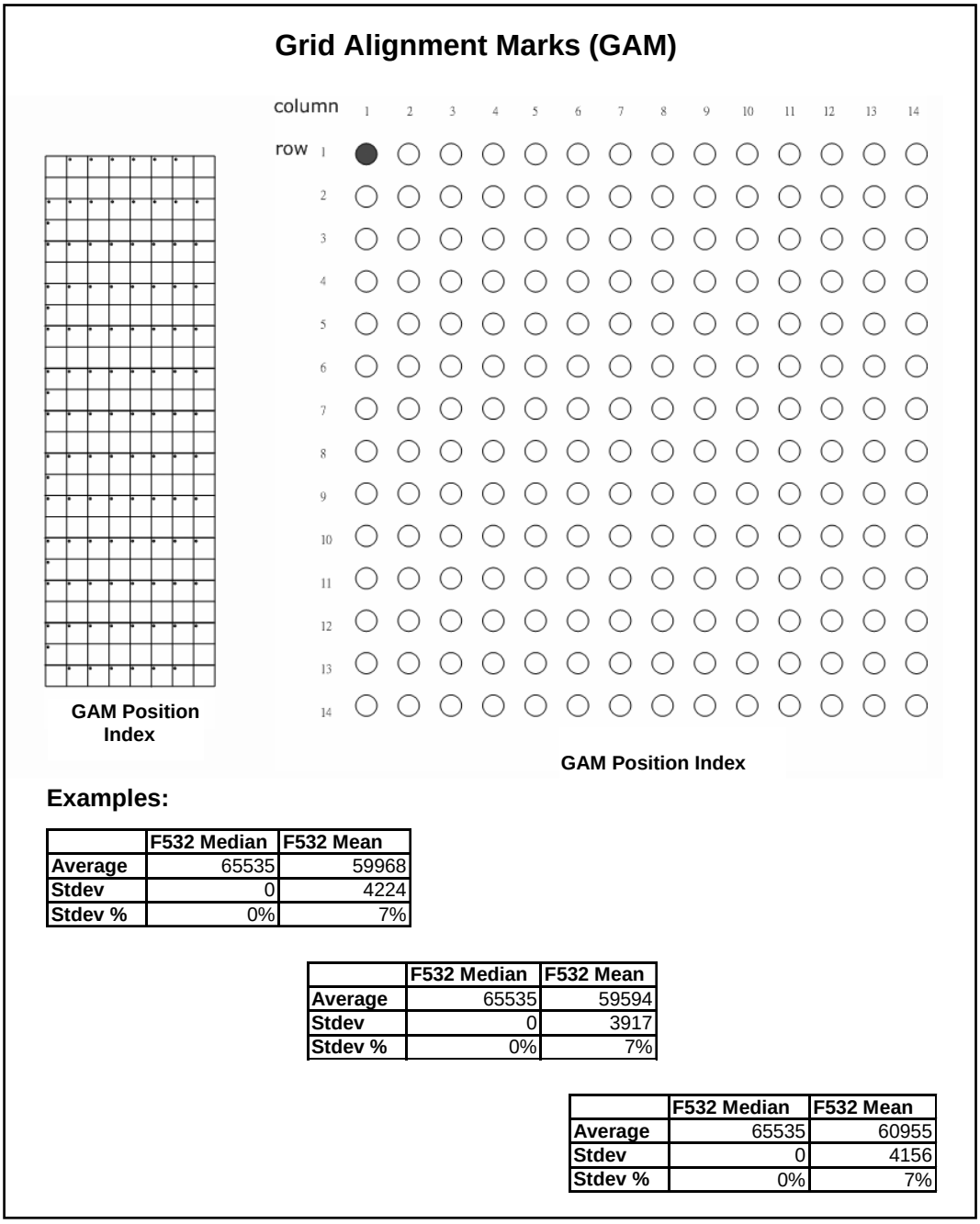

Figure 6: Example of Grid Alignment Marks (GAM)

Cy3 or Cy5 Intensity Ladder (C3_IL or C5_IL)

- Probe ID Numbers:
  Cy3 Intensity Ladder PH_c_0000002 to PH_c_0000009
  Cy5 Intensity Ladder PH_c_0000010 to PH_c_0000017
- Purpose:
  - To assist in adjusting the scanner settings
- Cy3 and Cy5 Intensity Ladder probes are a Cy3- or Cy5-labeled 60-mer oligonucleotide probes designed from an alien sequence that do not cross-hybridize with human targets. Eight dilutions, and three to six replications of each dilution of these features can be excited respectively by a 532 nm laser, or a 635 nm laser without hybridization.
- Instructions:

  1) Convert scanned data into Excel format.
  2) Sort data by column name: ID.
  3) Copy rows of data with Probe ID numbers above.
  4) Obtain the average of the 3-6 replicates in the "F532 Medium" and "F635 Medium" columns.

     NOTE: Occasionally, some spots will be missing or misaligned, so the intensity results can be removed for a more accurate average. (This is noted in Figure 8 with an asterisk [*].)

     Expected Outcome:
     When the suggested scanner settings that are listed in Step 6 are used, saturated or near-saturated signal should be observed for probes C3_IL PH_c_0000008 and C5_IL PH_c_0000016. Scanner settings should be adjusted so that the signal increases linearly from the lowest concentration unit in order to observe the saturation level.

Table 8, below, provides the ID number of the Cy3 & Cy5 Intensity Ladder (C3_IL & C5_IL) controls and detailed information about their content.

Table 8: C3_IL & C5_IL Probe Information

C3_IL	***C5_IL***	***Probe Concentration Unit***
PH_c_0000002	PH_c_0000010	0.39
PH_c_0000003	PH_c_0000011	0.78
PH_c_0000004	PH_c_0000012	1.56
PH_c_0000005	PH_c_0000013	3.125
PH_c_0000006	PH_c_0000014	6.25
PH_c_0000007	PH_c_0000015	12.50
PH_c_0000008 (=GAM)	PH_c_0000016	25.00
PH_c_0000009	PH_c_0000017	50.00

Figures 7 and 8, below, provide plotted and graphed examples of the results of Cy3 and Cy5 Intensity Ladder controls.

Cy3 Intensity Ladder (C3_IL)

ID	F532 Median
PH_c_0000002	24232
PH_c_0000002	25761
PH_c_0000002	17389
PH_c_0000002	25254
PH_c_0000002	29489
PH_c_0000002	22350
PH_c_0000003	43213
PH_c_0000003	3398
PH_c_0000003	42420
PH_c_0000003	23061
PH_c_0000003	19961
PH_c_0000003	34751
PH_c_0000004	65535
PH_c_0000004	65535
PH_c_0000004	65535
PH_c_0000004	65535
PH_c_0000004	65535
PH_c_0000004	65535
PH_c_0000005	65535
PH_c_0000005	65535
PH_c_0000005	65535
PH_c_0000005	65535
PH_c_0000005	65535
PH_c_0000005	65535
PH_c_0000006	65535
PH_c_0000006	65535
PH_c_0000006	65535
PH_c_0000006	65535
PH_c_0000006	65535
PH_c_0000006	65535
PH_c_0000007	65535
PH_c_0000007	65535
PH_c_0000007	65535
PH_c_0000007	65535
PH_c_0000007	65535
PH_c_0000007	65535
PH_c_0000008	65535
PH_c_0000008	65535
PH_c_0000008	65535
PH_c_0000008	65535
PH_c_0000008	65535
PH_c_0000009	65535
PH_c_0000009	65535
PH_c_0000009	65535
PH_c_0000009	65535
PH_c_0000009	65535
PH_c_0000009	65535

1. Take the average of the replicates

	F532 Median
PH_c_0000002	22413
PH_c_0000003	25734
PH_c_0000004	60404
PH_c_0000005	65535
PH_c_0000006	65535
PH_c_0000007	65535
PH_c_0000008	65535
PH_c_0000009	65535

2. Plot a graph

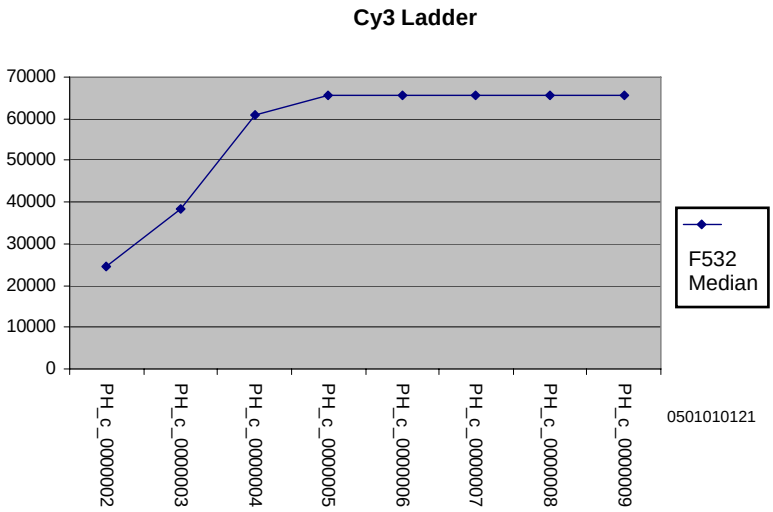

Figure 7: Example Results for Cy3 Intensity Ladder

Cy5 Intensity Ladder (C5_IL) II

ID	F635 Median	
PH_c_0000010	14342	
PH_c_0000010	11315	
PH_c_0000010	11770	
PH_c_0000010	8960	
PH_c_0000010	8380	
PH_c_0000010	56	*
PH_c_0000011	26350	
PH_c_0000011	23183	
PH_c_0000011	19701	
PH_c_0000011	17534	
PH_c_0000011	14222	
PH_c_0000011	12190	
PH_c_0000012	45665	
PH_c_0000012	42320	
PH_c_0000012	48153	
PH_c_0000012	42469	
PH_c_0000012	47566	
PH_c_0000012	49	*
PH_c_0000013	65535	
PH_c_0000013	65535	
PH_c_0000013	65535	
PH_c_0000013	65535	
PH_c_0000013	65535	
PH_c_0000013	41219	
PH_c_0000014	65535	
PH_c_0000014	65535	
PH_c_0000014	65535	
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PH_c_0000016	65535	
PH_c_0000016	65535	
PH_c_0000016	65535	
PH_c_0000017	65535	
PH_c_0000017	65535	
PH_c_0000017	65535	
PH_c_0000017	65535	
PH_c_0000017	65535	
PH_c_0000017	65535	

1. Take the average of the replicates

	F635 Median
PH_c_0000010	9137
PH_c_0000011	16841
PH_c_0000012	39727
PH_c_0000013	54621
PH_c_0000014	65535
PH_c_0000015	65535
PH_c_0000016	65535
PH_c_0000017	65535

2. Plot a graph

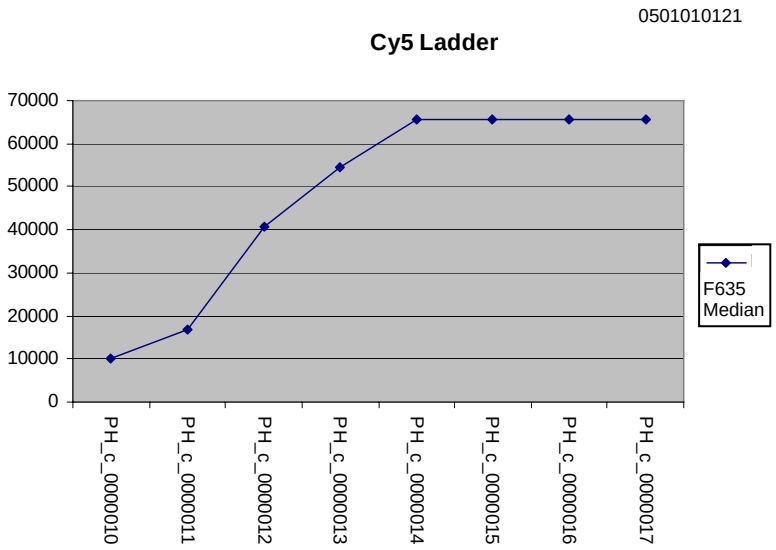

Figure 8: Example Results for Cy5 Intensity Ladder

Positive Controls

Intrinsic Hybridization Controls (IHC)

- Probe ID Number: PH_c_0000025
- Purpose:
  - To monitor the overall quality of the sample processing and hybridization efficiency
- IHCs contain mixtures of probes from 58 robustly expressed housekeeping genes that serve as built-in positive controls. The probe mixture is dispensed at a fixed concentration, and more than 95 IHC features are evenly distributed across the arrayed area. The hybridization signal intensity of IHCs gives the indication of overall quality of sample processing and hybridization efficiency.
- Instructions:

  1) Convert scanned data into Excel format.
  2) Sort data by column name: ID.
  3) Copy rows of data with Probe ID numbers above.
  4) Obtain the average of the 3-6 replicates in the "F532 Medium" and "F635 Medium" columns (see examples next page).

     NOTE: Occasionally, some spots will be missing or misaligned, so the intensity results can be removed for a more accurate average.

Intrinsic Hybridization Ladder (IHL)

- Probe ID Numbers: PH_c_0000026 to PH_c_0000033
- Purpose:
  - To monitor the overall quality of the sample processing and hybridization efficiency
- The IHL probes are dispensed in serial dilutions of IHC controls. The data extracted from IHL after target hybridization can be used to construct a hybridization intensity response curve depending on different dilutions of the probe mixture, which will be able to serve as an indicator to monitor the overall consistency of sample processing and hybridization stringency.
- Instructions:

  1) Convert scanned data into Excel format.
  2) Sort data by column name: ID.
  3) Copy rows of data with Probe ID numbers above.
  4) Obtain the average of the 3-6 replicates in the "F532 Medium" and "F635 Medium" columns (see examples next page).

     NOTE: Occasionally, some spots will be missing or misaligned, so the intensity results can be removed for a more accurate average.

     Expected Outcome:
     When the suggested scanner settings that are listed in Step 6 are used, saturated or near saturated signal should be observed for all IHC probes. Scanner settings should be adjusted so that the signal increases linearly from the lowest concentration IHL to the highest concentration level of IHL.

Table 9, below, provides the ID number of the IHL controls and detailed information about their content.

Table 9: IHL Probe Information		
Probe ID	**Probe Name**	**Probe Concentration Units**
PH_c_0000026	IHL_1	0.39
PH_c_0000027	IHL_2	0.78
PH_c_0000028	IHL_3	1.56
PH_c_0000029	IHL_4	3.13
PH_c_0000030	IHL_5	6.25
PH_c_0000031	IHL_6	12.50
PH_c_0000032	IHL_7	25.00
PH_c_0000033	IHL_8	50.00
PH_c_0000025	IHC	100.00

Figure 9, below, provides a plotted and graphed example of the results of the Intrinsic Hybridization Control and Ladder (IHC and IHL).

Intrinsic Hybridization Control (IHC) & Ladder (IHL)

1. Take the average of the replicates from the data

Table 8: IHL Probe Information

Probe ID	*Probe Name*	*Probe Concentration Units*
PH_c_0000026	IHL_1	0.39
PH_c_0000027	IHL_2	0.78
PH_c_0000028	IHL_3	1.56
PH_c_0000029	IHL_4	3.13
PH_c_0000030	IHL_5	6.25
PH_c_0000031	IHL_6	12.50
PH_c_0000032	IHL_7	25.00
PH_c_0000033	IHL_8	50.00
PH_c_0000025	IHC	100.00

		F635 Medium	F532 Medium
PH_c_0000026	IHL_1	2261	1963
PH_c_0000027	IHL_2	4238	3823
PH_c_0000028	IHL_3	7812	7465
PH_c_0000029	IHL_4	14013	13289
PH_c_0000030	IHL_5	24125	22614
PH_c_0000031	IHL_6	37162	34060
PH_c_0000032	IHL_7	53616	46739
PH_c_0000033	IHL_8	59969	54356
PH_c_0000025	IHL	57032	52282

2. Plot a graph

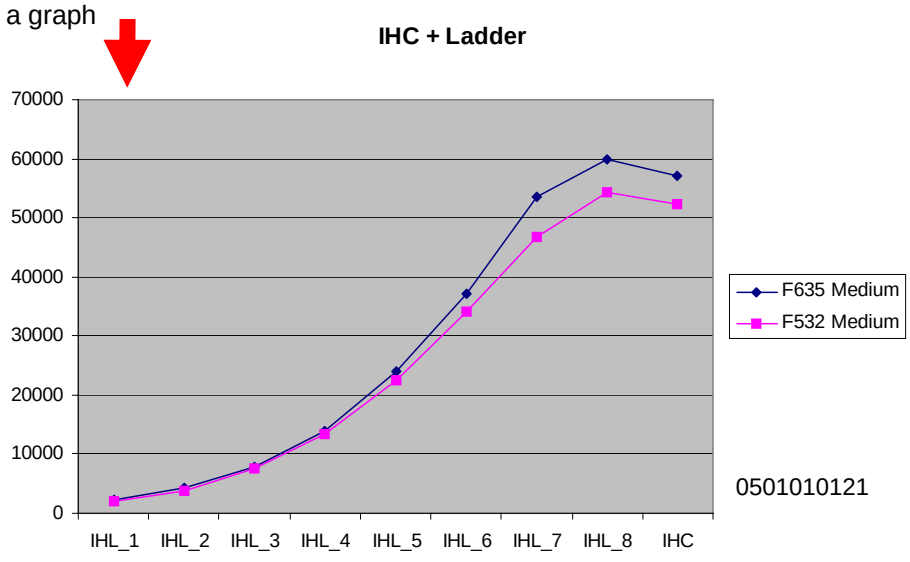

Figure 9: Example Results for Cy3 Intensity Ladder

Intrinsic Target Quality Control (ITQC)

- Probe ID Numbers: PH_c_0000038 to PH_c_0000065
- Purpose:
  - To monitor the length of the targets for assessment of the quality of sample integrity and processing
- Four probes per gene were selected from seven consistently expressed housekeeping genes. For each gene, one probe is designed from regions of 300-600 bp, 900-1200 bp, 1500-1800 bp, and 2100-2400 bp, respectively, from each 3' end of the transcript.
- Instructions:

  1) Convert scanned data into Excel format.
  2) Sort data by column name: ID.
  3) Copy rows of data with Probe ID numbers above.
  4) Obtain the average of the 3-6 replicates in the "F532 Medium" and "F635 Medium" columns (see examples next page).

  NOTE: Occasionally, some spots will be missing or misaligned, so the intensity results can be removed for a more accurate average.

  Expected Outcome:
  When good quality targets are used, signals from all the probes can be observed. It is common to observe higher signals from probes designed closer to the 3' end of the transcript.

Table 10, below, lists each ITQC gene number and corresponding name.

Table 10: ITQC Gene Number and Name	
ITQC Gene Number	***Gene Name***
C_ITQC2	Lysosomal-associated membrane protein 1
C_ITQC3	Calnexin
C_ITQC4	Ubiquitin specific protease 11
C_ITQC5	Damage-specific DNA binding protein 1
C_ITQC6	Ubiquitin-activating enzyme 28
C_ITQC7	Tripartite motif-containing 28
C_ITQC8	Actin Binding LIM protein 1

Table 11, below, provides ITQC probe locations and ID numbers.

Table 11: ITQC Probes								
	ITQC Gene No.	**C_ITQC2**	**C_ITQC3**	**C_ITQC4**	**C_ITQC5**	**C_ITQC6**	**C_ITQC7**	**C_ITQC8**
Location from 3" end	**300-600 nt**	**PH_c_0000038**	**PH_c_0000042**	**PH_c_0000046**	**PH_c_0000050**	**PH_c_0000054**	**PH_c_0000058**	**PH_c_0000062**
	900-1200 nt	**PH_c_0000039**	**PH_c_0000043**	**PH_c_0000047**	**PH_c_0000051**	**PH_c_0000055**	**PH_c_0000059**	**PH_c_0000063**
	1500-1800 nt	**PH_c_0000040**	**PH_c_0000044**	**PH_c_0000048**	**PH_c_0000052**	**PH_c_0000056**	**PH_c_0000060**	**PH_c_0000064**
	2100-2400 nt	**PH_c_0000041**	**PH_c_0000045**	**PH_c_0000049**	**PH_c_0000053**	**PH_c_0000057**	**PH_c_0000061**	**PH_c_0000065**

Figure 10, below, provides a plotted and graphed example of the results of the Intrinsic Target Quality Control (ITQC).

Intrinsic Target Quality Control (ITQC) I

	F635 Medium	F532 Medium
PH_c_0000038	3677	5437
PH_c_0000039	1129	1546
PH_c_0000040	602	778
PH_c_0000041	380	477
PH_c_0000042	7157	9197
PH_c_0000043	5533	6163
PH_c_0000044	3950	5646
PH_c_0000045	2688	2952
PH_c_0000046	1309	2365
PH_c_0000047	932	973
PH_c_0000048	396	969
PH_c_0000049	259	287
PH_c_0000050	1499	1648
PH_c_0000051	1849	1778
PH_c_0000052	590	679
PH_c_0000053	240	329
PH_c_0000054	1499	1844
PH_c_0000055	414	518
PH_c_0000056	199	290
PH_c_0000057	1591	1756
PH_c_0000058	3970	4764
PH_c_0000059	784	879
PH_c_0000060	476	584
PH_c_0000061	369	407
PH_c_0000062	545	957
PH_c_0000063	213	1580
PH_c_0000064	216	3425
PH_c_0000065	78	138

1. Take the average of the replicates from the data

2. Plot a graph

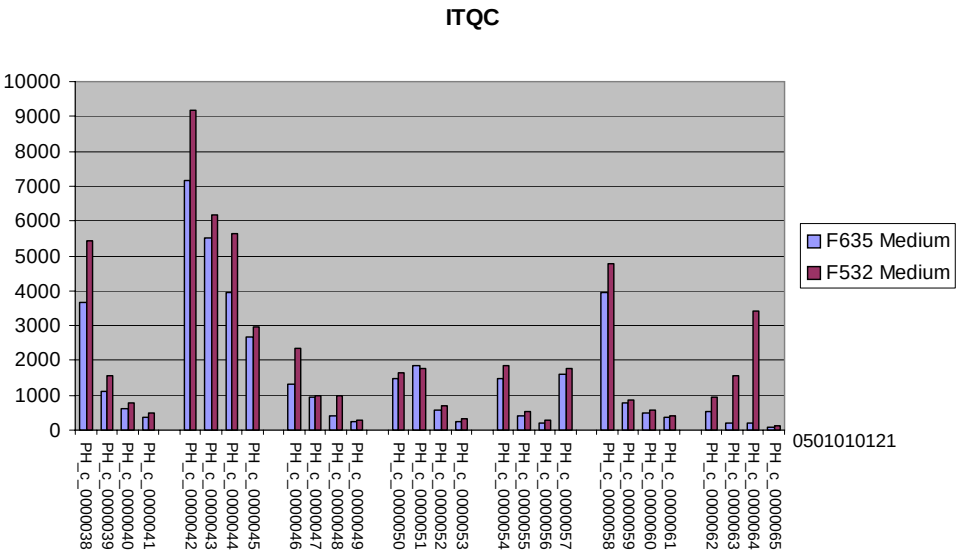

Table 11: ITQC Probes

	ITQC Gene No.	C_ITQC2	C_ITQC3	C_ITQC4	C_ITQC5	C_ITQC6	C_ITQC7	C_ITQC8
Location from 3" end	300-600 nt	PH_c_0000038	PH_c_0000042	PH_c_0000046	PH_c_0000050	PH_c_0000054	PH_c_0000058	PH_c_0000062
	900-1200 nt	PH_c_0000039	PH_c_0000043	PH_c_0000047	PH_c_0000051	PH_c_0000055	PH_c_0000059	PH_c_0000063
	1500-1800 nt	PH_c_0000040	PH_c_0000044	PH_c_0000048	PH_c_0000052	PH_c_0000056	PH_c_0000060	PH_c_0000064
	2100-2400 nt	PH_c_0000041	PH_c_0000045	PH_c_0000049	PH_c_0000053	PH_c_0000057	PH_c_0000061	PH_c_0000065

Figure 10: Example Results Intrinsic Target Quality Control (ITQC)

Figure 11, below provides a plotted and graphed example of the results of technical repeats of the Intrinsic Target Quality Control (ITQC) using one labeled sample on two arrays.

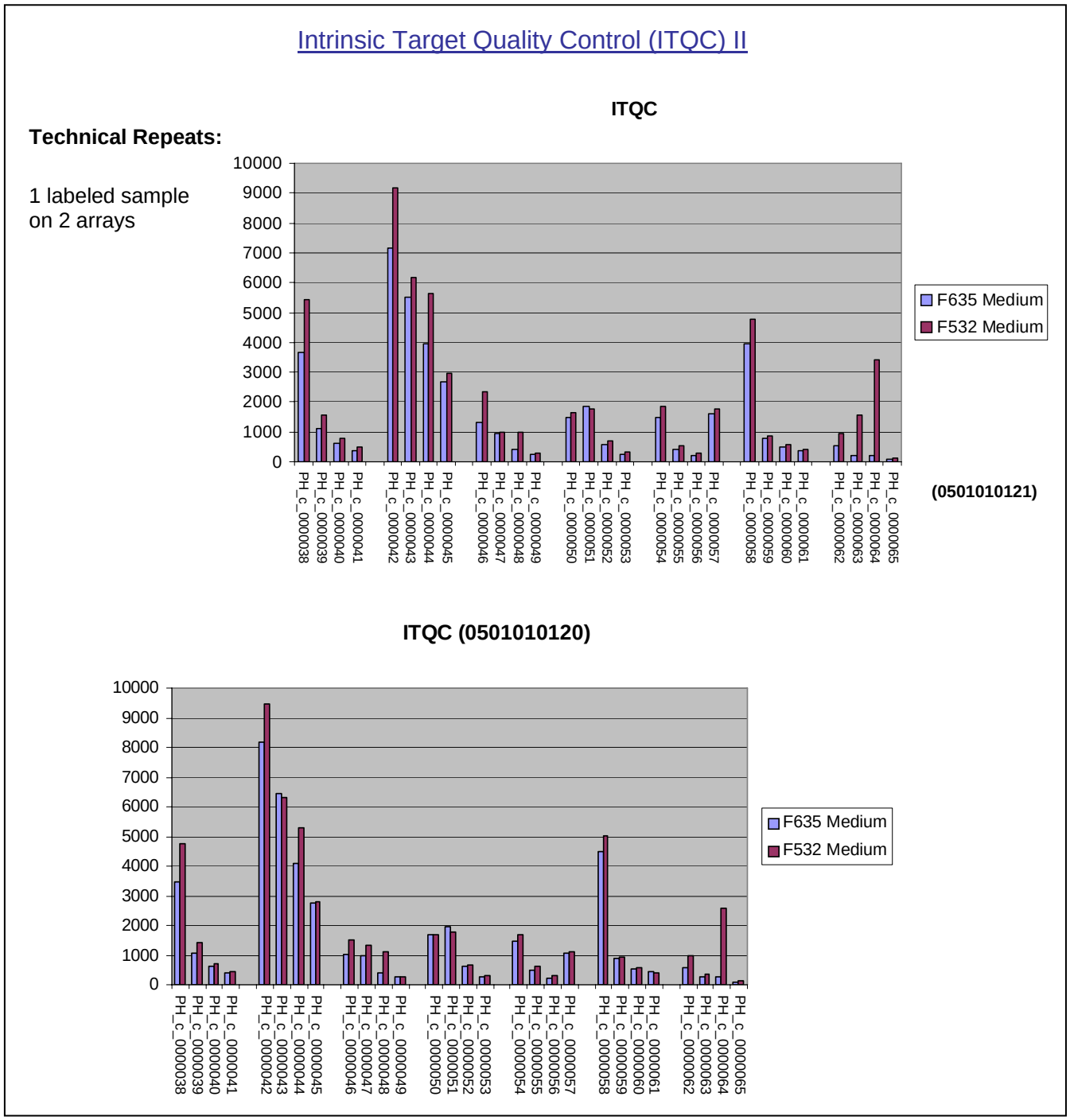

Figure 11: Example results of technical repeats of Intrinsic Target Quality Control (ITQC)

Figure 12, below provides a plotted and graphed example of the results of a dye-swap experiment performed using the Intrinsic Target Quality Control (ITQC).

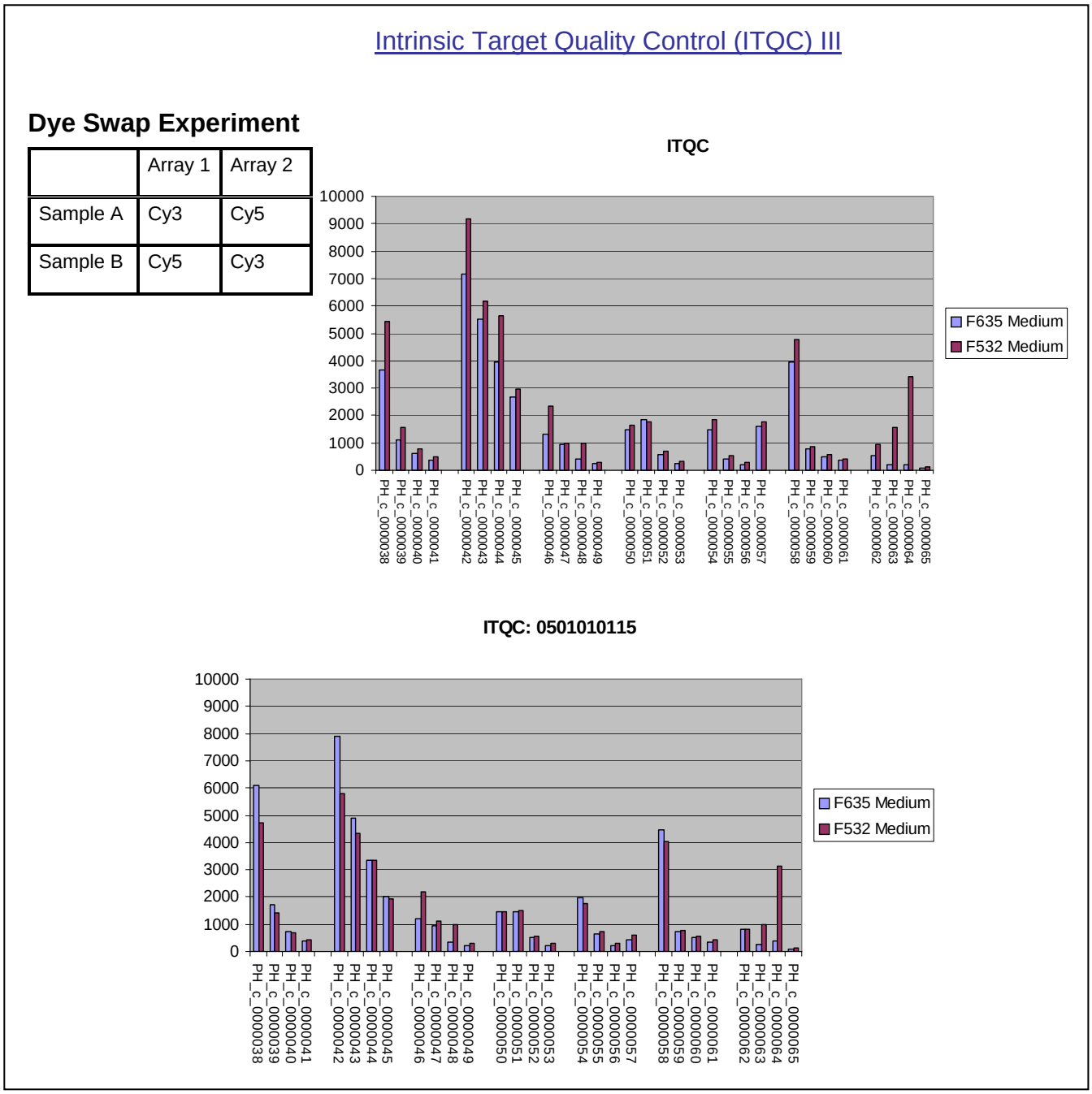

Figure 12: Example results of Dye-Swap Experiment using Intrinsic Target Quality Control (ITQC)

Negative or SPIKE-in Controls

Ambion ArrayControl™ RNA SPIKE: #1~8

- Probe ID Numbers: AM_c_0000015 to AM_c_0000022
- Purpose:
  - To monitor the specificity of the hybridization
  - To be used with spike-in positive controls to determine the sensitivity of the hybridization
  - To be used with spike-in controls to trouble-shoot the target preparation and labeling reactions
- Sequence information of ArrayControl™ RNA SPIKE: #1~8 from Ambion, Inc., has been licensed for use with the Human Microarray. Using the sequence information, eight 60-mer oligonucleotide probes are designed to compliment the respective RNA SPIKES. These probes are alien probe sequences that do not cross-hybridize with human genomic probes on the Human Microarray, and thereby provide a set of negative controls, which can be used as spike-in positive controls to determine the sensitivity of the hybridization process.
- Instructions:

  1) Convert scanned data into Excel format.

  2) Sort data by column name: ID.

  3) Copy rows of data with Probe ID numbers above.

  4) Obtain the average of the 3-6 replicates in the "F532 Medium" and "F635 Medium" columns (see examples next page).

     Expected Outcome:
     To learn more about the usage of RNA Spikes, access Applied Biosystem Ambion's Web site.

Table 12, below, lists the ArrayControl RNA SPIKE #1~8 probe ID numbers and their corresponding names.

Table 12: Ambion ArrayControl™ RNA SPIKE #1~8	
Probe ID #	***Ambion ArrayControl™ RNA SPIKE ID #***
AM_c_0000015	Array Control RNA SPIKE 1
AM_c_0000016	Array Control RNA SPIKE 2
AM_c_0000017	Array Control RNA SPIKE 3
AM_c_0000018	Array Control RNA SPIKE 4
AM_c_0000019	Array Control RNA SPIKE 5
AM_c_0000020	Array Control RNA SPIKE 6
AM_c_0000021	Array Control RNA SPIKE 7
AM_c_0000022	Array Control RNA SPIKE 8

ArrayControl™ RNA SPIKES and additional information can be obtained from Ambion, Inc., at:

www.ambion.com

Figure 13, below, provides a plotted and graphed example of the results of the Ambion Negative SPIKE-in Controls.

Ambion Negative SPIKE-in Control

1. Take the average of the replicates from the data

2 Plot a graph

	F635 Mean	B635 Mean	F532 Mean	B532 Mean
AM_c_0000015	52	49	125	88
AM_c_0000016	337	51	454	84
AM_c_0000017	64	52	148	91
AM_c_0000018	58	51	131	85
AM_c_0000019	87	51	174	84
AM_c_0000020	74	49	141	82
AM_c_0000021	55	49	158	82
AM_c_0000022	56	46	121	84

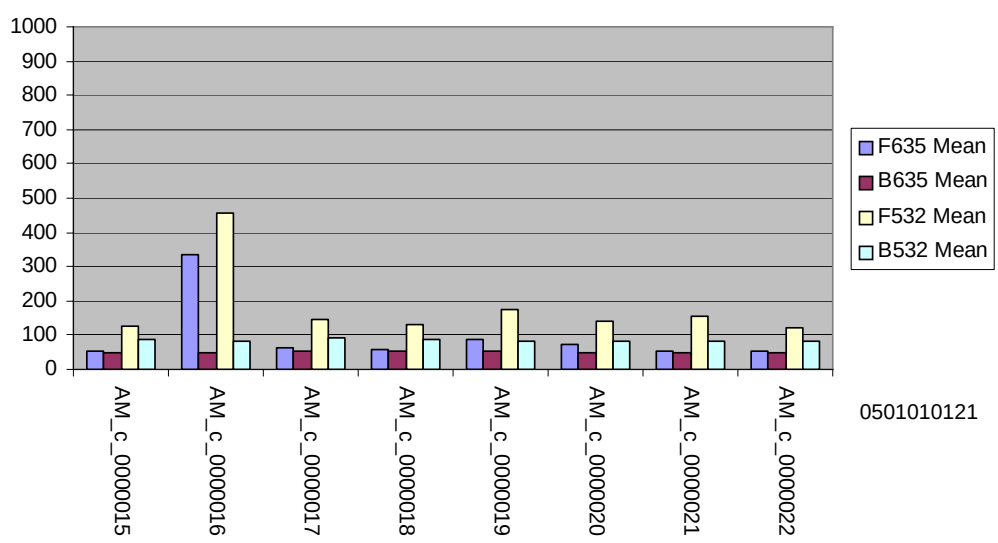

Figure 13: Example results of Ambion Negative Spike-in Controls

Negative Control/Extrinsic Target Quality Control (ETQC)

- Probe ID Numbers: PH_c_0000066 to PH_c_0000071
- Purpose:
  - To monitor the specificity of the hybridization
  - To be used with spike-in positive controls to determine the sensitivity of the hybridization
  - To be used with spike-in controls to trouble-shoot the target preparation and labeling reactions
- ETQC probes are alien probe sequences that do not cross-hybridize with human genomic probes on Human Microarray. The targets to these probes can be prepared separately as RNA containing a poly-A tail and spiked into the experimental RNA sample to monitor target preparation quality and determine the sensitivity of the hybridization reaction. Instructions:
- Instructions:

1) Convert scanned data into Excel format.
2) Sort data by column name: ID.
3) Copy rows of data with Probe ID numbers above.
4) Obtain the average of the 3-6 replicates in the "F532 Medium" and "F635 Medium" columns (see examples next page).

   Expected Outcome:
   These probes emit no or low signal. Targets matching to these probes can be obtained (see Table 13, below), and used in a manner similar to how they are used in the Ambion RNA Spike controls, above.

Probe ID: Clones ID* Reference

Table 13: Prob ID: Clones ID* Reference for ETQC Probes

Probe ID #	***Reference #***
PH_c_0000066~ ref:	NM_202341.1 (Entrez Gene ID: 842522)
PH_c_0000067~ ref:	NM_122927.2 (Entrez Gene ID: 833497)
PH_c_0000068~ ref:	NM_001036207.1 (Entrez Gene ID: 843854)
PH_c_0000069~ ref:	NM_104167.4 (Entrez Gene ID: 841722)
PH_c_0000070~ ref:	NM_129890.3 (Entrez Gene ID: 818930)
PH_c_0000071~ ref:	NM_106286.3 (Entrez Gene ID: 843969)

Figure 14, below provides a plotted and graphed example of the results of technical repeats of the Intrinsic Target Quality Control (ITQC) using one labeled sample on two arrays.

Negative Spike-in Controls

1. Take the average of the replicates from the data

2. Plot a graph

	F635 Mean	B635 Mean	F532 Mean	B532 Mean
PH_c_0000066	149	52	184	87
PH_c_0000067	64	50	121	85
PH_c_0000068	73	51	136	82
PH_c_0000069	54	50	115	80
PH_c_0000070	61	50	116	82
PH_c_0000071	52	47	118	85

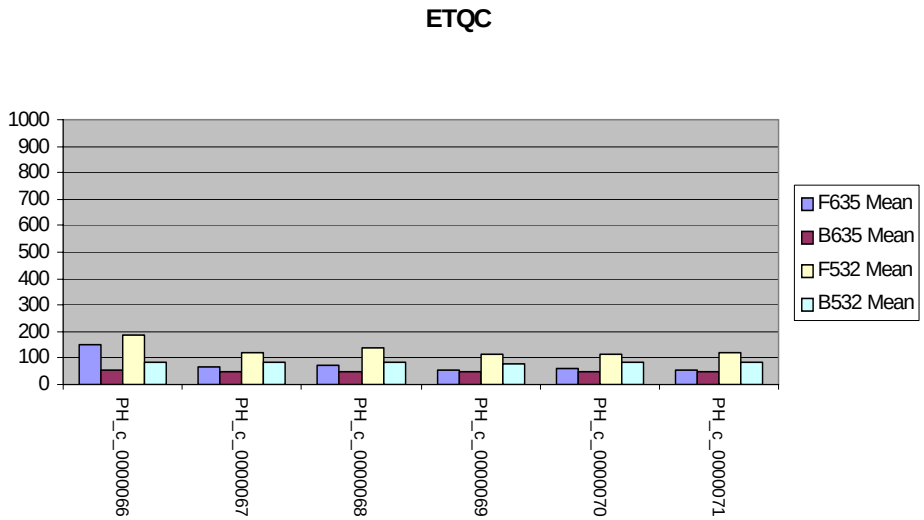

Figure 14: Example results of Negative Spike-in Controls

Additional information about the control probes is included on the Product Support CD, and on our Web site at:

www.eurogentec.com

# # # #